

Effect of flaxseed oil on acetaminophen-induced hepatotoxicity in male rats

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ABSTRACT

The goal of this study was to explore if flaxseed oil (FSO) could protect male rats from hepatotoxicity caused by acetaminophen (APAP). Feed intake (FI), feed efficiency ratio (EFR) and body weight gain % (BWG %), as well as serum levels of liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase, (ALP)) used to investigate these impacts. A liver histopathological investigation was also carried out. *Methods:* Fifty male Wister rats were divided into five groups at random: Group 1 is the negative group (Cont -), while Group 2 is the positive group (Cont +). The other three groups; rats were received oral gavages of FSO (1.5, 3 and 4.5ml/Kg/day) for 30 days and received the single dose of APAP (3 g/kg) at day 30 1 h before FSO respectively. *Results:* The results demonstrated that pretreatment with FSO (1.5, 3, and 4.5 ml/kg b.wt.) for 30 days improved FI, EFR, BWG percent, and lowered high serum levels of liver enzymes in hepatotoxic rats compared to the Cont+ group. The histopathological alterations generated by APAP were reduced in liver sections of rats given flaxseed oil. *Conclusion:* The results suggest that FSO induces potent hepatoprotective in acetaminophen hepatotoxic rats. Flaxseed oil consumption may be useful for people who have liver disorders, according to this study.

Keywords: Flaxseed oil, Liver enzymes, Oxidative stress, acetaminophen

1. INTRODUCTION

Liver is considered the body's largest internal organ (Hinton et al., 2009). It is an essential organ with multiple functions, as controlling the metabolism of carbohydrate, proteins, and fats (Pandit et al., 2012). It also detoxifies the body from many harmful substances (Gordillo et al., 2015). Hepatotoxicity is pathophysiological changes of the liver due to infections, diseases, or chemicals that affect the structure and function of the liver, which may result in many acute and chronic liver dysfunctions such as hepatitis, cirrhosis, jaundice, fibrosis etc (Bjornsson, 2016). Acetaminophen (N-acetyl-p-aminophenol) (APAP), also known as paracetamol, is a commonly abused over-the-counter (OTC) drug that accounts for approximately 50% of the 2000 cases of acute liver failure in the United States each year, with acetaminophen accounting for 37% and other medications accounting for 13% (William, 2017).



According to the Saudi Journal of Gastroenterology, Saudi Arabia has a high prevalence of liver illnesses. It was estimated to be 8,451,000 in 2017, with a projection of 12,534,000 by 2030 (Khalid et al., 2018). Natural resources got the attention to evaluate their use and effectiveness in preventing many diseases, to minimize the unwanted side effect of chemicals based drugs (Dash et al., 2007). Due to FSO high quantities of omega-3 fatty acid and linolenic acid, flaxseed oil is gaining appeal as a functional food ingredient (Gorkem et al., 2019).

Flaxseed oil (FSO) provides an excellent ω -3 fatty acid source solely from the vegetarian diet and phytoestrogenic lignans, which have anti-inflammatory and antioxidant effects and may help to reduce the risk of heart disease, atherosclerosis, diabetes, autoimmune, arthritis, cardiovascular disease, liver injuries, cancer, and neurological disorders (Mian *et al.*, 2017). As a result, the goal of this research is to see if FSO can prevent acetaminophen-induced hepatotoxicity in male rats.

2. MATERIAL AND METHODS

Material

Drugs and Chemicals

Acetaminophen (APAP) was obtained from Alnahdi pharmacy. Sigma, in the United States, provided all of the high-grade chemicals and kits.

Flaxseed oil

Flaxseed oil was obtained from Abazeer organic food stories, Jeddah, Saudi Arabia.

Animals

The animals were purchased from experimental unit of King Fahd Medical Research Center; King Abdulaziz University. This study was carried out in King Fahd Medical Research Center, KAU, from January 2020 till October 2020.

Methods

Induction of hepatotoxicity and experimental design

The 50 male Wister rats, which weighed around 180 ± 10 g, before being employed in this study, all animals were given a one-week acclimation period in the animal house. The rats were housed in a standard laboratory setting with a temperature of 22 ± 3 degrees Celsius, a relative humidity of 50-55 percent, and a 12-hour light/dark cycle. All of the animals were fed a conventional nutritionally balanced diet and were provided free access to water (Haimeur et al., 2019). After acclimatization period, the rats were distributed randomly into five equal groups of ten rats each according to the following

Group 1 (Cont -): Rats were fed on the basal diet for 29 days and received orally saline.

Group 2(Cont +): Rats were fed on the basal diet for 29 days and received the single dose of APAP (3 g/kg) at day 30 according to Mustafa *et al.* (2015).

Group 3: Rats were received oral gavages of FSO 1.5 ml/Kg/day for 29 days and received the single dose of APAP (3 g/kg) at day 30 1 h before FSO.

Group 4: Rats were received oral gavages of FSO 3 ml/Kg/day for 29 days and received the single dose of APAP (3 g/kg) at day 30 1 h before FSO. Malik *et al.* (2017)

Group 5: Rats were received oral gavages of FSO 4.5 ml/Kg/day for 29 days and received the single dose of APAP (3 g/kg) at day 30 1 h before FSO.

Rats were sacrificed under light ether anesthesia for all groups after 30 days of experiment. Blood samples were obtained in heparinized capillary tubes and centrifuged for 15 minutes at 3000 rpm after being kept at room temperature for two hours. For subsequent testing, the separated serum was stored at 20°C. Histopathological investigations of the liver were carried out.

Determination of body weight gain % and feed efficiency ratio

Throughout the experiment FI per group, BWG%, and FER were measured according to the method of Chapman et al., (1959).

Determination of serum biomarkers

According to Reitman and Frankel (1957) the activities of liver enzymes in serum AST, ALT and ALP were measured.

Histopathological examination

Routine techniques were used to prepare and stain liver tissue samples from all groups using hematoxylin and eosin.

Statistic

All results were analyzed by SPSS ver. 24, by using ANOVA, values were expressed as mean \pm SD, P-value <0.05 considered significance.

3. RESULTS

Body weight (initial and final) and BWG % of hepatotoxic male rats pretreated with FSO (1.5, 3, and 4.5 ml/Kg/day) were shown in Table 1. The data revealed that there was no significant difference in the experimental groups' in initial body weight. Rats intoxicated with APAP (3 g/kg) showed significant declines in final b.wt and BWG percent compared to Cont (-). In comparison to Cont (+), there was a significant increase in final b.wt and BWG percent in pretreated groups with FSO at three dosage levels intoxicated by APAP (3 g/kg).

Table 1 Effect of flaxseed oil (FSO) on the initial weight, final weight and body weight gain percent against hepatotoxicity induced by acetaminophen (APAP)-in male rats.

Groups	Initial weight (g)	Final weight (g)	Body weight gain (%)
Group (1) Negative control	188.43 \pm 1.45 ^a	224.32 \pm 2.03 ^a	19.05 \pm 0.17 ^a
Group (2) Positive control(APAP)	184.22 \pm 1.11 ^a	177.75 \pm 1.37 ^c	- 3.51 \pm 0.01 ^c
Group (3) FSO 1.5 ml/Kg/day+ APAP	187.21 \pm 1.97 ^a	192.31 \pm 1.11 ^b	2.72 \pm 0.11 ^c
Group (4) FSO 3 ml/Kg/day+ APAP	184.32 \pm 1.21 ^a	212.44 \pm 1.43 ^b	15.26 \pm 0.07 ^b
Group (5) FSO 4.5 ml/Kg/day+ APAP	182.63 \pm 1.03 ^a	220.13 \pm 2.58 ^a	20.53 \pm 0.13 ^a

Values are expressed as mean \pm SD.

Values with different superscript letters within a column are significantly different at $P < 0.05$.

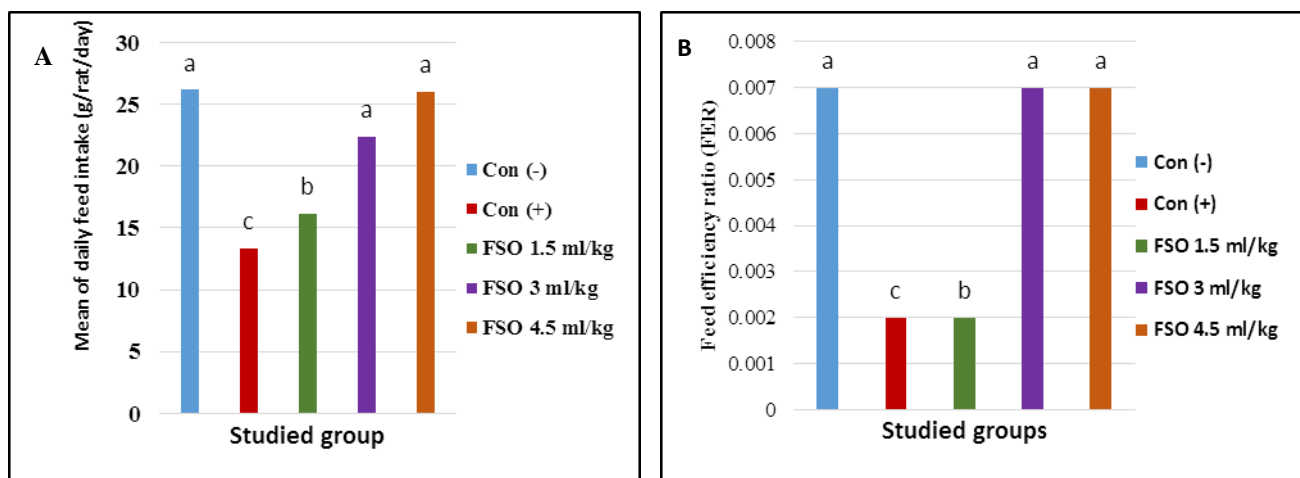


Figure 1 Effect of flaxseed oil on feed intake (A), feed efficiency ratio (FER) (B) against hepatotoxicity induced by acetaminophen (APAP) - in male rats. Values are expressed as mean \pm SD. Values with different superscript letters within a column are significantly different at $P < 0.05$.

Figure 1 (A & B) showed the pretreatment effect of different doses of FSO (1.5, 3 and 4.5 ml/Kg/day) on FI and FER in hepatotoxic rats. The results indicated that, there was a significant decrease in FI between APAP group and the Cont. (-) by 49.10%.

Significant increase ($p < 0.05$) was observed in FI in intoxicated rats pretreated orally with FSO at the three dosage levels (1.5, 3 and 4.5 ml/Kg/day) by 20.95%, 67.87% and 95.20 % respectively, as compared to the Cont. (+) (Figure 1A.). Figure (1B) Showed that there was a significant decrease ($p < 0.05$) in FER between APAP group and the negative control group. Intoxicated rats pretreatment with FSO at all dosages 1.5, 3, and 4.5 ml/Kg/day had significantly higher FER compared with the Cont (+) group.

From data recorded in Table (2) it might be recognized that intoxicated rats by APAP (3 mg/kg) had significant ($P < 0.05$) increases the serum liver enzyme activities (AST, ALT and ALP) by 47.27, 57.05 and 13.71%, respectively as compared to the Cont(-). Administration of FSO (3and 4.5 ml/kg) to rats intoxicated with APAP exhibit significant decreases ($P < 0.05$) in all the elevated liver marker as compared to the Cont (+).

Table 2 Effect of flaxseed oil on the serum level of liver function enzymes against hepatotoxicity induced by acetaminophen (APAP) - in male rats.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Group (1) Negative control	43.62 \pm 2.33 ^c	37.54 \pm 1.26 ^c	97.39 \pm 2.41 ^c
Group (2) Positive control (APAP)	64.24 \pm 2.16 ^a	58.96 \pm 1.76 ^a	110.75 \pm 2.09 ^a
Group (3) FSO 1.5 ml/Kg/day+ APAP	60.74 \pm 3.12 ^a	54.44 \pm 2.23 ^a	113.63 \pm 2.11 ^a
Group (4) FSO 3 ml/Kg/day+ APAP	50.28 \pm 2.43 ^b	41.21 \pm 3.54 ^b	102.26 \pm 1.52 ^b
Group (5) FSO 4.5 ml/Kg/day+ APAP	45.65 \pm 2.63 ^c	38.84 \pm 2.43 ^c	98.61 \pm 2.83 ^c

Values are expressed as mean \pm SD.

Values with different superscript letters within a column are significantly different at $P < 0.05$.

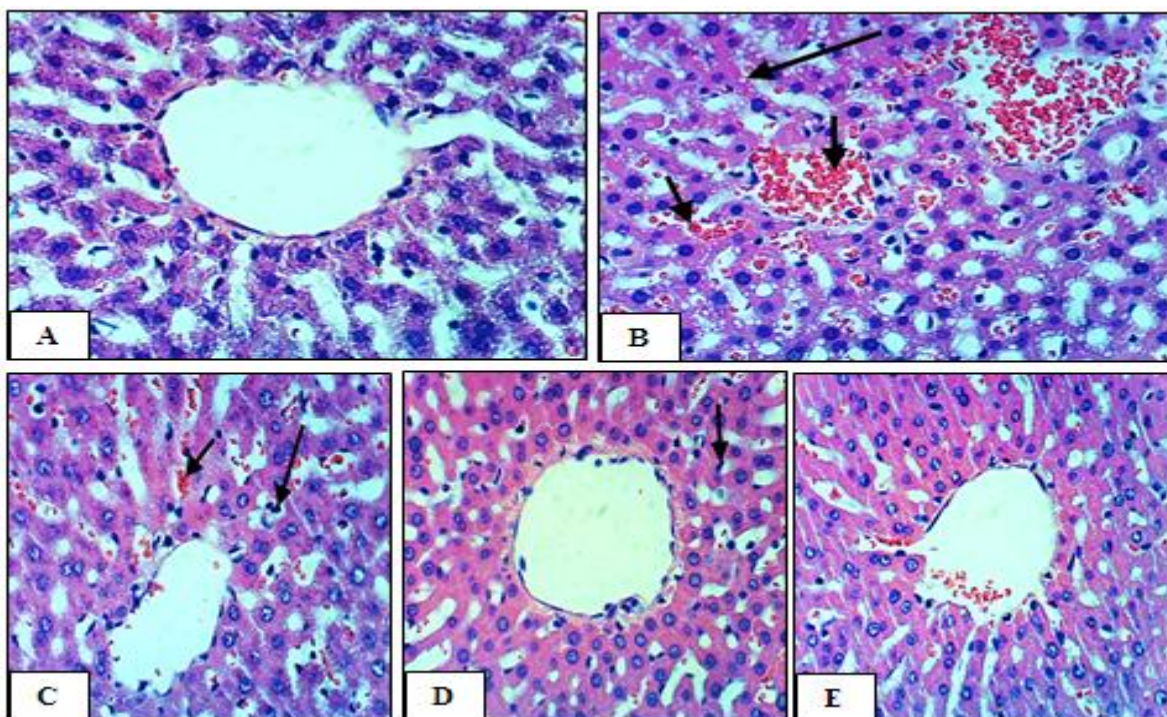


Figure 2 Photomicrographs of H&E-stained liver sections in several groups.

Histopathological results

Hepatic lobule histology in Cont. (-) rats was normal, with normal hepatic sinusoids and hepatocytes (Fig 2A). Administration of APAP to rats induced inflammatory cell infiltration, hepatic necrosis and hepatocytes apoptosis (Fig 2B). Liver tissue of rats given

FSO (1.5ml/kg) + APA. Revealed hepatic sinusoids congestion and activation of Kupffer cells (Fig 2C). Slight congestion of hepatic sinusoids are shown in group of rat orally given FSO (3ml/kg) + APAP (Fig 2D). In-group received FSO (4.5ml/kg) + APAP revealed apparently normal architecture of hepatic tissue (Fig 2E).

4. DISCUSSION

Liver disorders continue to be severe health issues, and managing liver disease remains a challenge for modern medicine. Hepatotoxicity is a serious metabolic condition that can lead to death (Patel et al., 2008). Hepatic injury arises as a result of its multifaceted roles. The most prevalent reason for stopping the development of a novel medicine is drug-induced liver damage (DILI) (David & Hamilton, 2010). Because acetaminophen (APAP) is a dose-dependent hepatotoxicant, overdose induced hepatotoxicity is a popular and clinically relevant experimental *in vivo* model (Jaeschke et al., 2011). When used in a single or many large dosages, or after a long period of use, it has been shown to induce toxicity (Siemionow et al., 2016). Increased apoptosis, cyclooxygenase-2 production, reactive metabolite release, and glutathione depletion are all symptoms of APAP toxicity in the liver (McGill et al., 2012).

The preventative role of FSO against APAP-induced hepatotoxicity was studied in this research. The findings revealed that consuming FSO orally reduced APAP's hepatotoxicity. The levels of a few key biochemical indicators in the blood were used as liver damage diagnostic markers. Body weight is frequently the most sensitive indicator of the detrimental effects of chemical toxicants and xenobiotics. When comparing the rat group given APAP to the Cont. (-) group in terms of FI and BWG percent, the rat group given APAP showed a significant reduction in final weight and BWG percent. These findings were on the same line with (Farag et al., 2017; Merdana et al., 2019; Hegazy et al., 2021). The fall in body weight of hepatotoxic rats could be owing to a decrease in feed intake caused by APAP poisoning, which caused the rats to lose their appetite. Furthermore, the favorable effect of antioxidant administration on APAP poisoning in terms of body weight seen in this study backs up previous findings by Malik et al., (2017), who indicated that antioxidants content of FSO neutralizes the harmful effect of CCl₄. Selim et al., (2018) demonstrated that FSO significantly increased BWG% and final body weight compared to the Cont(+) group.

The APAP is a direct hepatotoxin that causes steatosis and centrilobular necrosis in the liver (Olaleye et al., 2014). The use of enzyme levels such as AST and ALT in the assessment of liver injury by APAP is common. The enzyme is released into circulation as a result of necrosis or membrane damage, and it can be identified in the blood. AST converts alanine to pyruvate and glutamate, which is then released in a similar manner. High AST values can indicate viral hepatitis-related liver damage, as well as myocardial infarction and muscular injury. The ALT is more liver-specific and, as a result, a more accurate indication of liver illness. Cellular leakage and a lack of cell membrane functional integrity are indicated by elevated blood enzyme levels in the liver (Madhumitha, 2010; Ismet et al., 2013). Hepatic cell function is linked to serum alkaline phosphatase (ALP). Increased synthesis of ALP in the presence of rising biliary pressure causes an increase in blood ALP levels (Ismet et al., 2013).

Histopathological observations of the liver of APAP- administered rat's revealed presence of karyomegaly, binucleated cells and sporadic cell necrosis. Fibrous connective tissue proliferation in the portal area, interlobular septa and hepatic capsule was evident. Hyperplasia of the bile duct epithelium was very clear as well. These results were in harmony with the previous data reported by previous findings of Jarsiah et al., (2017). It also has been reported that APAP causes apoptosis in liver (Shahid and Subhan, 2014). The FSO medication reduced AST, ALT, and ALP levels significantly during the experiment (30 days). The current findings were consistent with those of Abdou and Hassan, (2014), who found that the FSO reduced serum liver enzyme levels. These changes could be related to the antioxidant content of FSO, which contains high levels of tocopherols and beta-carotene, which may help to scavenge free radicals or reduce rises in serum aminotransferase during the early stages of liver injury (Akhtar et al., 2013).

These histological findings matched those of Malik et al., (2017), who found that pretreatment with FSO improved the shape of hepatic cells substantially. Our study's biochemical findings were corroborated by histological findings in liver sections. The livers of rats treated with FSO in a dose of 4.5ml/Kg/day exhibited practically entirely normal structure. This could be explained by FSO's antioxidant activity, which can be linked to its omega-3 and omega-6 fatty acid contents, as well as the presence of phytoestrogenic lignans, particularly secoisolariciresinol diglucoside (Aljedaani et al., 2020).

5. CONCLUSION

It may be inferred that FSO reduces the liver damage caused by APAP. It works through the antioxidant system to protect all biological and biochemical parameters, as well as histopathological changes.

Ethical approval

The protocol was approved by the Vice Deanship of Graduate Studies and Scientific Research, Faculty of Human Sciences and Design, KAU, no (191060231).

Funding

This study has not received any external funding.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are presented in the paper.

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